**APPENDIX 4-2: Mixture and Abiotic Stressors Analysis for Chlorpyrifos**

The current risk assessment focuses on chlorpyrifos and its degradates. The approach is based on data describing the environmental fate, estimated exposure concentrations, and potential toxicity of chlorpyrifos. There is uncertainty in the potential effects of chlorpyrifos because the approach does not quantitatively consider the presence of other chemicals in the environment of the assessed species. Of particular concern would be cases where the presence of other chemicals results in an increase in the toxicity of chlorpyrifos, thus, the effects characterization may under predict the potential effects of chlorpyrifos on listed species. Although there are some data to indicate that this may occur, effects of chemical mixtures on the expected toxicity of chlorpyrifos are not consistent across species, taxa or concentrations of the stressors. This section discusses the available information on environmental exposures to chemical mixtures that include chlorpyrifos as well as toxicity data describing whether chemicals mixed with chlorpyrifos may result in expected toxicity based on additivity, synergism or antagonism. Toxicity data available for exposures involving technical grade and formulated chlorpyrifos are described in the effects characterization section.

## **Environmental Mixtures**

Mixtures may be present in ESA-listed species’ habitats following the offsite transport of pesticides and other chemical constituents (*e.g.*, other active ingredients, inerts, adjuvants, *etc*.) through the use of co-formulated products or tank mixes at individual or multiple locations. Species and their habitats exposed to pesticide mixtures may be at greater risk of adverse effects than when exposed to single pesticides. Recent review articles indicate that additivity (*i.e.*, concentration- or response-addition) is the appropriate default assumption when considering mixture toxicity (Cedergreen 2014; Belden *et al*. 2007). Experimental results from numerous studies indicate that exposure to OP-containing mixtures produces additive and synergistic toxicity, as measured by activity of the neurological enzyme acetylcholinesterase (AChE), in several taxa groups including mammals, fish, birds, amphibians, and aquatic insects.

Due to the large number of pesticides that may be present in a species’ habitat at any one time, it is not feasible to estimate exposure concentrations for all possible mixture combinations. Furthermore, it is not practical to test the toxicity of every mixture combination in every ESA-listed species or appropriate surrogate. However, qualitative assessments of mixture toxicity can be made using expected exposures, principles of additive toxicity, and known toxic responses in published scientific literature. The mixtures line of evidence is considered qualitatively using available product labels, usage information, monitoring data, and taxa-specific toxicity data.

**1.1.1 Composition of mixtures in terrestrial and aquatic environments**

Chemical mixtures are present in terrestrial and aquatic environments. This may be due to application of multiple chemicals contained in formulations and tank mixtures of formulations, resulting in a direct application to the terrestrial environment or spray drift of the mixtures onto adjacent terrestrial, wetland or aquatic habitats. Chemical mixtures may also be present in aquatic systems due to transport from upstream applications.

Most formulated products contain multiple chemicals (referred to as “inert ingredients”), and may also contain multiple active ingredients (a.i.). A listing of inert ingredients that are formulated with pesticide a.i. is available on the EPA website[[1]](#footnote-1). Chlorpyrifos products that are co-formulated with other a.i.’s generally include pyrethroids (*e.g.,* bifenthrin, cyfluthrin, *etc*.), however, there are some cattle ear tag products that contain chlorpyrifos and diazinon (both OP insecticides). In addition to being co-formulated, a.i.’s are routinely used together as tank mixtures (*i.e.*, multiple products used at the same time) in agricultural practices, as a means to enhance the effectiveness of the ingredient a.i., as well as to treat multiple pest pressures at the same time and avoid the need to conduct multiple applications. Pesticide labels routinely provide instructions for tank mixtures, indicating to the applicator which chemicals can and cannot be used with the product. **Appendix 1-4** includes the information on chlorpyrifos labels that involves recommended tank mixtures.

Unless a pesticide label explicitly prohibits tank mixing of specific a.i.’s or formulated products, other products not identified on a label may be applied at the same time. A review of available pesticide application sources, such as California’s Pesticide Use Report (CAPUR), reports from the United States Department of Agriculture’s National Agricultural Statistics Service, and the GfK Kynetec database (market research data), depict applications of multiple pesticide a.i.’s to a field at the same time. **SUPPLEMENTAL INFORMATION 1** provides an analysis of the available CAPUR data for 2008-2012, depicting the top 25 a.i.’s with which chlorpyrifos was applied. All a.i.’s were applied with chlorpyrifos less than 10% of the time chlorpyrifos was applied. When chlorpyrifos was applied as a mixture (98,351 times), 6% of the time it was with chlorthal-dimethyl (benzenedicarboxylic acid herbicide), lambda-cyhalothrin (pyrethroid insecticide), and imidacloprid (neonicotinoid insecticide), 5% of the time with abamectin (insecticide) and 4% of the time with oxyfluorofen (diphenyl ether herbicide). **Figures S1** and **S2** in **SUPPLEMENTAL INFORMATION 1** also display the ratio of the application rate of the a.i.’s to the application rate of chlorpyrifos. Of the chemicals discussed above, only the chlorthal-dimethyl:chlorpyrifos combination has the majority of the applications (85%) where chlorthal-dimethyl is applied at an application rate at or greater than that of chlorpyrifos. Of the remaining four a.i.’s, the majority of the applications had a ratio where the application rate of chlorpyrifos was 1-10x that of the a.i. . This suggests chemicals that are applied in a tank mix with chlorpyrifos are not always mixed at the maximum labeled application rates.

Monitoring data from state and federal agencies have indicated that multiple pesticides often co-occur in aquatic habitats located throughout the US. Studies conducted by the United States Geological Survey, under the National Water Quality Assessment program, have routinely detected the presence of multiple chemicals in surface water and groundwater samples (Gilliom *et al*., 1999, 2006; Gilliom, 2007).

USGS summarized the composition of pesticide mixtures observed in surface water samples collected throughout the US during the 1990s. The analysis determined that herbicides were the most commonly detected pesticides within agricultural areas, with atrazine and its degradates being the most frequently detected (found in 2/3 of all samples taken from streams with agricultural landcovers representing their watersheds). More than 50% of the stream samples had ≥5 different a.i.’s. Atrazine and metolachlor were the most commonly detected mixture in agricultural watersheds, followed by atrazine, prometon and metolachlor (USGS 1999[[2]](#footnote-2)). A review of NAWQA data collected between 1992 and 2001 showed that atrazine, metolachlor, and cyanazine were the most frequently detected herbicides in agricultural watersheds, while diazinon, chlorpyrifos and carbaryl were the most frequently detected insecticides (USGS 2006)[[3]](#footnote-3). Mixture composition varied over time, with different compositions of chemicals and relative amounts measured. **Table 4-2.1** includes the most frequently detected mixtures of pesticide a.i.’s in streams with agricultural watersheds. It should be noted that these data are based on non-targeted sampling collected throughout the US.

**Table 4-2.1. The most common unique mixtures of pesticides and degradates found in stream waters with agricultural watersheds. From USGS 2006.**

|  |  |  |
| --- | --- | --- |
| **Number of chemicals in mixture** | **Chemicals present** | **Frequency of detection in agricultural streams****(percentage of time )** |
| 2 | Atrazine, Metolachlor | 77 |
| Atrazine, Deethylatrazine\* | 77 |
| Atrazine, Simazine | 64 |
| Atrazine, Prometon | 50 |
| Prometon, Simazine | 41 |
| Deethylatrazine, Metolachlor | 69 |
| Deethylatrazine, Simazine | 57 |
| 3 | Atrazine, Deethylatrazine, Prometon | 48 |
| Atrazine, Prometon, Simazine | 41 |
| Atrazine, Diazinon, Simazine | 16 |
| Atrazine, Diazinon, Prometon | 10 |
| Diazinon, Prometon, Simazine | 9 |
| Atrazine, Deethylatrazine, Metolachlor | 69 |
| Atrazine, Deethylatrazine, Simazine | 57 |
| Atrazine Metolachlor, Simazine | 57 |
| 4 | Atrazine, Deethylatrazine, Metolachlor, Simazine | 52 |
| Atrazine, Deethylatrazine, Metolachlor, Prometon | 45 |
| Alachlor, Atrazine, Deethylatrazine, Metolachlor | 42 |
| Atrazine, Deethylatrazine, Prometon, Simazine | 39 |
| Atrazine, Metolachlor, Prometon, Simazine | 38 |
| Atrazine, Diazinon, Prometon, Simazine | 9 |
| 5 | Atrazine, Deethylatrazine, Metolachlor, Prometon, Simazine | 37 |
| Alachlor, Atrazine, Deethylatrazine, Metolachlor, Prometon | 33 |
| Alachlor, Atrazine, Deethylatrazine, Metolachlor, Simazine | 33 |
| Atrazine, Cyanazine, Deethylatrazine, Metolachlor, Simazine | 33 |
| Alachlor, Atrazine, Deethylatrazine, Prometon, Simazine | 26 |
| Atrazine, Deethylatrazine, Metolachlor, Simazine, Tebuthiuron | 19 |
| Atrazine, Deethylatrazine, Prometon, Simazine, Tebuthiuron | 16 |
| Atrazine, Diazinon, Metolachlor, Prometon, Simazine | 8 |
| Atrazine, Deethylatrazine, Diazinon, Prometon, Simazine | 8 |
| Atrazine, Carbaryl, Diazinon, Prometon, Simazine | 2 |

\*degradate of atrazine

**1.1.2 Influence of other chemicals on chlorpyrifos toxicity**

Several studies were located in the open literature that evaluated the potential toxicological interactions of chlorpyrifos and other pesticides or environmental contaminants. According to the available data, other chemicals may combine with chlorpyrifos to produce synergistic, additive, or antagonistic toxic effects. If chemicals that show such effects are present in the environment in combination with chlorpyrifos the toxicity of chlorpyrifos may be increased, offset by other environmental factors, or even reduced by the presence of antagonistic contaminants if they are also present in the mixture. The variety of chemical interactions presented in the available data set suggest that the toxic effect of chlorpyrifos, in combination with other pesticides used in the environment, can be a function of many factors including, but not necessarily limited to: (1) the exposed species, (2) the co-contaminants in the mixture, (3) the ratio of chlorpyrifos and co-contaminant concentrations, (4) differences in the pattern and duration of exposure among contaminants, and (5) the differential effects of other physical/chemical characteristics of the environment.

Acute mammalian toxicity data for formulated products that contain chlorpyrifos and other a.i.’s are also submitted by registrants as part of the six-pack data and **Appendix 1-11** provides an analysis of the toxicity data from the co-formulated products in comparison to the technical chlorpyrifos formulation. The “co-formulated” products included the following a.i.’s: bifenthrin, zeta-cypermethrin, cyfluthrin, gamma-cyhalothrin, and lambda cyhalothrin, however, the analysis is of limited utility because the data are limited to mammals (often from up-down studies) and are generally insufficient for making a direct comparison of the toxicity of chlorpyrifos alone vs. the formulated product toxicity. Therefore, this section will focus on the data available from the open literature.

***Other AChE inhibitors:*** Macek (1975) provides acute toxicity values for 29 two-chemical mixture tests conducted with blue gill (*lepomis macrochirus*) to discern possible trends for combinations that are less than additive (antagonistic), additive (expected toxicity-no interaction), or greater than additive (synergism). While chlorpyrifos was not included, there were several organophosphate and carbamate a.i.’s tested. In this study, there were combinations of AChE inhibitors that resulted in higher toxicity than expected (Malathion/Parathion; Baytex/Malathion; Sevin/Malathion; EPN/Malathion; and Diazinon/Parathion), while others demonstrated the expected toxicity, (Sevin/methyl parathion; Sevin/Methyl Parathion). Another study with Coho salmon (*Oncorhynchus kisutch*-E114293; Laetz *et al*., 2009) tested binary mixtures of all possible combinations of five AChE inhibitors (diazinon, malathion, chlorpyrifos, carbaryl, and carbofuran) and all combinations produced toxicity that was either additive or synergistic. In an in-vitro study involving chinook salmon olfactory gland extracts, Scholz *et al.,* (2006) found that a mixture of carbaryl, carbofuran, diazoxon, maloxon and chlorpyrifos-oxon (carbamate and OP insecticides) resulted in the expected decrease in AChE activity, indicating additive toxicity for the mixture. While this summary is not a comprehensive review of all available AChE inhibitor mixture data, the available information suggests that there is some uncertainty with assuming the toxicity of AChE inhibitors will always be strictly additive.

For mammals, Gordon *et al*. (2006), assessed the interaction between the anticholinesterase insecticides chlorpyrifos and carbaryl using hypothermia and acetylcholinesterase (AChE) inhibition as toxicological endpoints. In this study, core body temperature was monitored by radiotelemetry in adult rats administered chlorpyrifos at doses ranging from 0 to 50 mg/kg and carbaryl at doses of 0–150 mg/kg and the change in core temperature (Temperature index: TI) over a 12-h period was quantified. Plasma and brain AChE activity were measured 4-h after dosing with chlorpyrifos, carbaryl, and mixtures in separate groups of rats. Effects of mixtures of chlorpyrifos and carbaryl in 2:1 and 1:1 ratios on the TI were examined and the data analyzed using a statistical model designed to assess significant departures from additivity for chemical mixtures. The temperature index (TI) response to a 2:1 ratio of chlorpyrifos: carbaryl was significantly less than that predicted by additivity, however, the TI response to a 1:1 ratio of chlorpyrifos: carbaryl was not significantly different from the predicted additivity. For the AChE analysis, there was a was a dose-additive interaction for the inhibition of brain AChE for the 2:1 ratio, but an antagonistic effect for the 1:1 ratio. The 2:1 and 1:1 mixtures had an antagonistic interaction on plasma AChE. Overall, the departures from additivity for the physiological (*i.e.,* temperature) and biochemical (*i.e.,* AChE inhibition) endpoints for the 2:1 and 1:1 mixtures studies did not coincide as expected. An interaction between chlorpyrifos and carbaryl appears to depend on the ratio of compounds in the mixture as well as the biological endpoint.

***Atrazine:*** Anderson and Lydy (2002) demonstrated that atrazine (10-200 µg/L), in combination with diazinon (0.9-4.3 µg/L), chlorpyrifos (0.0003-0.0427 µg/L) or methyl parathion (0.3-2.1 µg/L), resulted in an increase in toxicity of the OP insecticide to *Hyalella azteca*. Tested concentrations were as follows: atrazine, 10-200 µg/L; diazinon, 0.9-4.3 µg/L; chlorpyrifos (0.0003-0.0427 µg/L); and methyl parathion 0.3-2.1 µg/L. The magnitude of the increase in toxicity to exposed organisms was related to the concentration of atrazine. At a concentration of 40 µg/L, atrazine increased the LC50 of chlorpyrifos by a factor of 1.6, but did not increase the toxicity of diazinon or methyl parathion. At an atrazine concentration of 80 µg/L, the toxicity of chlorpyrifos, diazinon and methyl-parathion increased by factors of 2.0, 2.0 and 1.7, respectively. At an atrazine concentration of 200 µg /L, the toxicities of chlorpyrifos, diazinon and methyl-parathion increased by factors of 2.8, 3.0 and 2.9, respectively. Concentrations of atrazine ≤10 µg/L did not increase the toxicity of the three OPs. The same authors also exposed house flies (*Musca domestica)* to atrazine and the three OPs (separately) via topical exposures. Atrazine did not alter the expected toxicity of the OPs to the house fly.

***Piperonyl butoxide (PBO):*** PBO is co-formulated with pyrethroid insecticides as a synergist, acting by inhibiting cytochrome P450, which prevents metabolism of pesticides. Ankley and Collyard (1995) demonstrated that PBO reduced the toxicity of diazinon, chlorpyrifos, and azinphos-methyl (all OP insecticides that require activation to their oxon forms) to *H. azteca* and *Chironomus tentans*. A reduction in toxicity was not observed for dichlorovos, which does not work through activation to an oxon. For diazinon and PBO (46.9-375 µg/L) exposures to *H. azteca*, the toxicity of diazinon was reduced by a factor of 5. For *C. tentans* exposed to PBO (125-1000 µg/L) and diazinon at concentrations 5 times the LC50 for diazinon alone (10.7 µg/L), no toxicity was observed. For chlorpyrifos, PBO (at 46.9-187 µg/L) also effectively reduced the toxicity to *H. azteca* with at least some survival occurring at insecticide concentrations an order of magnitude greater than the initial LC50 of 0.04 µg/L and for *C. tentans*, the toxicity was markedly reduced with PBO concentrations ranging from (350-900 µg/L)*. L. variegatus* did not follow the same trend as the other two test species. When PBO (312-2500 µg/L) was applied in combination with diazinon, the toxicity of diazinon did not decrease, but rather was slightly higher.

***Prochloraz (fungicide):*** Johnston *et al.* (1994) examined potential impacts of prochloraz (180 mg/kg-bw) on the effects of diazinon (4.3 mg/kg-bw) on AChE inhibition in the plasma of Hybrid Red-legged partridge (*Alectoris rufa + A. graeca + A. chukar*). The authors reported no significant difference in plasma AChE inhibition of birds exposed to the combination compared to birds exposed to diazinon only. The authors also examined potential impacts of prochloraz on AChE inhibition of chlorpyrifos (9 mg/kg-bw) and dimethoate (3 mg/kg-bw). The results of the chlorpyrifos experiment were similar to those of diazinon, *i.e.,* no significant impact to AChE inhibition of chlorpyrifos alone. In contrast, in the dimethoate experiment, AChE inhibition was significantly lower in birds exposed to the combination when compared to dimethoate alone.

***Alphacypermethrin, bromopropylate, carbendazim, mancozeb:*** Jacobson *et al*. (2004, E93040) exposed mice to chlorpyrifos alone and as a mixture with four other pesticides commonly associated with food commodities. For the single agent exposure to chlorpyrifos, the author reported a slight but statistically significant decrease in hematocrit and an increase in thyroid weights (males only) in the 0.15 mg/kg exposure group as compared to the controls. A slight decrease in plasma cholinesterase over controls was also reported in this group for males only (graphically displayed but no numerical data provided); females had plasma cholinesterase levels three times as high as males in control and all treatment groups with no significant difference between control and treatment groups. No differences in brain acetylcholinesterase were reported or any other physiological or behavioral changes in the single a.i. treatment group. Exposure to chlorpyrifos in any of the mixture combinations did not increase the magnitude of brain or plasma cholinesterase inhibition compared to exposure to chlorpyrifos alone.

***Mercury:*** A series of studies on the joint action of methylmercury with chlorpyrifos were previously reviewed (U.S. HHS, 2006). In Steevens and Benson (2001), the joint action was studied in acute toxicity tests with the amphipod, *Hyalella azteca*, with concentrations ranging from 0.125-4 times the median lethal concentration with a constant concentration of chlorpyrifos (0.42 nM; 0.147 ppb). The joint action of methylmercury (MeHg) and chlorpyrifos (CYP) was additive as judged by the fit of the (MeHg +chlorpyrifos) dose response curve to the fit of the additive dose response curve. Further study by Steevens and Benson (1999, 2000) was conducted to determine the effects to AChE when adult *hyallea azteca* were exposed for 48-hours to either MeHg alone, chlorpyrifos alone, or a mixture of the either 30nM MeHg and 0.04 nM CYP or 150nM MeHg + 0.14 nM CYP. CYP alone inhibited AChE in a dose dependent manner at ≥0.14 nM and as expected MeHg did not have an effect on the enzyme. The mixture of 150 nM MeHg (10 ppb) and 0.14 nM CYP (0.049 ppb) appears to have a protective effect as the inhibition seen with this mixture was significantly less than what was observed with 0.14 nM CYP alone. Steevens and Benson further investigated the accumulation and elimination of these compounds and, overall, the work suggests the joint action of MeHg and CYP with regard to lethality may have been due to increased accumulation of MeHg in the organisms in combination with enhanced deactivation of CYP due to MeHg, or possibly due to the toxicity of the complex itself.

In summary, the available data indicate that other pesticides or environmental contaminants in combination with chlorpyrifos may show additive, synergistic, or antagonistic toxicity in different taxa exposed to different chemical combinations. Therefore, the assumption that the toxicity of AChE inhibitors is strictly additive does not always hold across species, across endpoints, and may also be influenced by the ratio of compounds in the mixture, and other environmental factors.

## **Abiotic Stressors**

Potential impacts of environmental conditions on the effects of chlorpyrifos are discussed qualitatively. Environmental factors that are known to alter the toxicity of a chemical include, pH, temperature, and low oxygen content. Evidence is available that suggest that changes in temperature can enhance the susceptibility of some taxa to OPs. However, evidence was not available on changes in pH or bacterial/viral prevalence affecting toxicity to OPs. Multiple experimental results from separate studies indicate that increases in temperature can result in more pronounced toxic effects from OPs compared to exposures at non-elevated temperatures in some taxa, particularly freshwater fish and aquatic invertebrates (Mayer and Ellersieck 1986; Osterauer and Kohler 2008; Laetz *et al.* 2014). Less is known about the responses of other taxa following exposure to the three OPs under elevated temperature conditions.

Acute lethality bioassays with OPs show a distinct, robust relationship between toxicity (measured by 96 h LC50s) and temperature (Mayer and Ellersieck, 1986). The experiments have been conducted with several species of fish and OPs including bluegill sunfish (phosmet, parathion, malathion, trichlorfon), rainbow trout (phosmet, chlorpyrifos, trichlorfon), yellow perch (azinphos methyl), Atlantic salmon (trichlorfon), and brook trout (trichlorfon). Most pesticides show a 2- to 4-fold increase in toxicity for each 10 ◦C rise in temperature. Therefore, elevated temperatures in habitats of aquatic invertebrates and fish are considered qualitatively as adverse impacts on listed aquatic species. Future enhancements to this line of evidence may include a review of 303-d listed surface waters for temperature within the action area.

**References**

Anderson TD and Lydy MJ (2002) Increased Toxicity to Invertebrates Associated with a Mixture of Atrazine and Organophosphate Insecticides. Environmental Toxicology and Chemistry. 21 (7): 1507-1514.

Ankley GT, Collyard SA (1995). Influence of Piperonyl Butoxide on the Toxicity of Organophosphate Insecticides to Three Species of Freshwater Benthic Invertebrates. *Comp. Biochem, Physiolol.* Vol. 110C (2) pp 149-155.

Belden, JB, Gillion, RJ, Lydy, MJ. 2007. How well can we predict the toxicity of pesticide mixtures to aquatic life? Integrated Environmental Assessment and Management 3(3): 364-372.

Cedergreen N. 2014. Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicology. PLoS ONE 9(5): e96580.

Gordon CJ, Herr DW, Gennings C, Graff JE, McMurray M, Stork L, Coffey T, Hamm A, Mack CM (2006) Thermoregulatory Response to an Organophosphate and Carbamate Insecticide Mixture: Testing the Assumption of Dose-Additivity. Toxicology Vol. 217 (1) pp 1-13 (E87642)

Jacobson H, Ostergaard G, Lam HR, Pouslon, ME, Frandsen H, Ladefoged, O, Meyer, O (2004) Repeated dose 28-day oral toxicity study in Wistar rats with a mixture of five pesticides often found as residues in food: alpha-cypermethrin, bromopropylate, carbendazim, chlorpyrifos and mancozeb. Food and Chemical Toxicology Vol. 42 pp 1269–1277 (E93040)

Johnston G, Walker C, Dawson, A. (1994) Interactive effects between EBI fungicides (prochloraz, propiconazole and penconazole) and OP insecticides (dimethoate, chlorpyrifos, diazinon and malathion) in the hybrid red-legged partridge. Environ. Toxicol. Chem. Vol. 13 (4). pp 615–620

Laetz CA, Baldwin DH, Collier TK, Hebert V, Stark JD, Scholz NL. (2009) The Synergistic Toxicity of Pesticide Mixtures: Implications for Risk Assessment and Conservation of Endangered Pacific Salmon. Environmental Health Perspectives. Vol. 117 (3). pp 348-353. (E114293)

Laetz CL, Baldwin DH, Herbert V, Stark JD, Scholz NL. 2014 Elevated temperatures increase the toxicity of pesticide mixtures to juvenile coho salmon. Aquatic Toxicology 146:38-44.

Mayer FL, Ellersieck MR. 1988. Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. U.S. Fish and Wildlife Service Publication number 160.

Macek, KJ (1975). Acute Toxicity of Pesticide Mixtures to Bluegills. Bulletin of Environmental Contamination and Toxicology. Vol 14 (6) pp 648-650

Osterauer R., Kohler H. 2008. Temperature-dependent effects of the pesticide thiaclopyrid and diazinon on the embryonic development of zebrafish (Danio rerio). Aquatic Toxicology 86: 485-494.

Scholz, NL, Truelove, NK, Labenia, JS, Baldwin DH, Collier TK (2006). Dose-additive inhibition of chinook salmon acetylcholinesterase activity by mixtures of organophosphate and carbamate insecticides. Environmental Toxicology and Chemistry. Vol. 25 (5) pp 1200–1207

Steevens JA, Benson WH (2000). Interactions of chlorpyrifos and methyl mercury: a mechanistic approach to assess chemical mixtures. Marine Environmental Research. Vol. 50 pp 113-117 (E56639)

Steevens JA, Benson WH (1999). Toxicological interactions of chlorpyrifos and methyl mercury in the amphipod, Hyalella azteca. [*Toxicol Sci.*](http://www.ncbi.nlm.nih.gov/pubmed/10630569) Vol. 52(2):168-77 (E72763)

U.S. Department of Health and Human Services (US HHS) (2006). Interaction Profile For: Chlorpyrifos, Lead, Mercury, and Methylmercury. US HHS-Agency for Toxic Substances and Disease Registry. Authors: Pohl H, Colman J. <http://www.atsdr.cdc.gov/interactionprofiles/IP-11/ip11.pdf>

**SUPPLEMENTAL INFORMATION 1. Table S1. CDPR Mixture Data for Chlorpyrifos, 2008-2012**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Rank | Chemical | Count | App Rate (lbs/A) | Ratio AI:Chlorpyrifos App Rates | % Mixture Apps | % ChlorpyrifosApps |
| Min | Avg | Max | Min | Avg | Max |
| 1 | CHLORTHAL-DIMETHYL | 6,282  | 4.69E-06 | 2.68E+00 | 9.66E+01 | 9.40E-06 | 1.46E+01 | 7.01E+04 | 6.39% | 4.87% |
| 2 | LAMBDA-CYHALOTHRIN | 6,162  | 3.15E-07 | 3.07E-02 | 3.85E+00 | 4.90E-04 | 9.70E-02 | 8.20E+00 | 6.27% | 4.78% |
| 3 | IMIDACLOPRID | 6,018  | 3.01E-08 | 1.93E-01 | 9.88E+01 | 1.39E-03 | 4.38E-01 | 1.07E+03 | 6.12% | 4.67% |
| 4 | ABAMECTIN | 4,527  | 3.92E-08 | 1.67E-02 | 2.46E+00 | 2.47E-05 | 1.51E-01 | 5.79E+02 | 4.60% | 3.51% |
| 5 | OXYFLUORFEN | 4,333  | 2.11E-03 | 3.13E-01 | 7.43E+00 | 4.17E-03 | 5.67E-01 | 6.00E+02 | 4.41% | 3.36% |
| 6 | ESFENVALERATE | 4,101  | 2.60E-04 | 4.55E-02 | 1.05E+00 | 2.74E-04 | 3.89E-02 | 5.91E+00 | 4.17% | 3.18% |
| 7 | INDOXACARB | 3,714  | 3.21E-05 | 6.22E-02 | 2.19E-01 | 2.51E-04 | 1.47E-01 | 3.97E+00 | 3.78% | 2.88% |
| 8 | PROPIONIC ACID | 3,508  | 1.82E-05 | 2.24E-01 | 1.89E+01 | 2.20E-03 | 2.19E-01 | 1.21E+01 | 3.57% | 2.72% |
| 9 | PROPYLENE GLYCOL | 3,141  | 8.67E-07 | 7.60E-02 | 4.70E+00 | 6.64E-05 | 8.09E-02 | 3.14E+00 | 3.19% | 2.44% |
| 10 | (S)-CYPERMETHRIN | 2,377  | 1.55E-03 | 4.01E-02 | 4.97E-01 | 3.86E-03 | 7.22E-01 | 1.54E+03 | 2.42% | 1.84% |
| 11 | DIETHYLENE GLYCOL | 2,349  | 1.80E-04 | 8.12E-02 | 9.23E+00 | 5.37E-04 | 8.83E-02 | 5.94E+00 | 2.39% | 1.82% |
| 12 | HEXYTHIAZOX | 1,842  | 1.09E-06 | 1.49E-01 | 5.11E+00 | 5.36E-04 | 1.30E-01 | 1.06E+01 | 1.87% | 1.43% |
| 13 | NALED | 1,610  | 1.25E-02 | 1.16E+00 | 1.47E+01 | 6.25E-03 | 1.25E+00 | 1.51E+01 | 1.64% | 1.25% |
| 14 | DIMETHOATE | 1,590  | 1.69E-04 | 4.70E-01 | 4.95E+00 | 2.54E-03 | 1.11E+00 | 2.38E+02 | 1.62% | 1.23% |
| 15 | GAMMA-CYHALOTHRIN | 1,566  | 1.13E-03 | 9.00E-03 | 1.07E-01 | 6.43E-03 | 1.80E-02 | 2.78E-02 | 1.59% | 1.22% |
| 16 | BETA-CYFLUTHRIN | 1,530  | 1.80E-03 | 1.78E-02 | 2.99E-01 | 6.85E-05 | 6.35E-02 | 8.42E-01 | 1.56% | 1.19% |
| 17 | PROPARGITE | 1,491  | 8.00E-03 | 2.19E+00 | 2.09E+01 | 4.01E-03 | 1.78E+00 | 4.61E+01 | 1.52% | 1.16% |
| 18 | SPIROMESIFEN | 1,491  | 2.41E-06 | 1.84E-01 | 4.95E+00 | 1.93E-03 | 2.48E-01 | 7.79E+00 | 1.52% | 1.16% |
| 19 | PYRIPROXYFEN | 1,435  | 1.25E-07 | 1.01E-01 | 1.08E+00 | 7.28E-05 | 5.08E-02 | 1.62E+00 | 1.46% | 1.11% |
| 20 | SPINOSAD | 1,416  | 8.61E-07 | 9.99E-02 | 3.91E+00 | 3.05E-05 | 2.51E-01 | 1.34E+01 | 1.44% | 1.10% |
| 21 | METHOXYFENOZIDE | 1,380  | 1.47E-03 | 2.23E-01 | 5.65E-01 | 1.01E-02 | 2.63E-01 | 2.67E+01 | 1.40% | 1.07% |
| 22 | ACETAMIPRID | 1,354  | 2.15E-06 | 7.23E-02 | 4.50E+00 | 5.90E-04 | 7.99E-02 | 1.80E+00 | 1.38% | 1.05% |
| 23 | FERROUS SULFATE | 1,321  | 3.29E-04 | 1.07E-02 | 1.40E-01 | 6.56E-04 | 1.52E-02 | 1.39E-01 | 1.34% | 1.03% |
| 24 | GLYPHOSATE, POTASSIUM SALT | 1,319  | 8.41E-03 | 1.47E+00 | 2.21E+01 | 4.93E-02 | 2.74E+00 | 8.48E+01 | 1.34% | 1.02% |
| 25 | GLYPHOSATE, ISOPROPYLAMINE SALT | 1,314  | 1.51E-05 | 1.92E+00 | 5.34E+01 | 2.66E-02 | 3.55E+01 | 2.02E+04 | 1.34% | 1.02% |



**Figure S-1. CALDPR Mixture Rate Data** Error bars represent range of 5th percentile application rate to 95th ]]percentile application rate for ai.

**Figure S-2. Ratio of AI Application Rate to Chlorpyrifos Application Rate**

1. http://www.epa.gov/opprd001/inerts/ [↑](#footnote-ref-1)
2. http://pubs.usgs.gov/circ/circ1225/ [↑](#footnote-ref-2)
3. http://pubs.usgs.gov/circ/2005/1291/pdf/circ1291.pdf [↑](#footnote-ref-3)